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# 'NUCLEOPHILIC REACTIONS IN CARBOHYDRATE CHEMISTRY'

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## ABSTRACT

This report describes work in which the lesser used nucleophiles, $N_3^-$, $SCN^-$, and $RS^-$, have been used on carbohydrate molecules.

Methyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannoside with sodium azide in boiling 2-methoxyethanol gave methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altroside (I). Reaction on the corresponding anhydro-alloside gave predominantly methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altroside (II) with a lesser amount of the 3-azido-3-deoxy-D-glucose isomer. All three azido sugars were characterised by reduction to the corresponding, known, amino sugars.

The azido-altrosides have been converted into sulphonate esters, which have been reduced to the corresponding amino, O-sulphonate derivatives. Various attempts were made to convert these compounds into 2,3-imino compounds. The use of hydrazine in the presence of Raney Ni was successful, and methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-imino-α-D-mannoside has been prepared, and converted into the corresponding 2,3-acetimido, and 2,3-benzimido derivatives. Use of sodium methoxide on the amino, O-sulphonates derived from (I) and (II) has led to crystalline products of unknown structure.

Direct $S_N2$ replacement of secondary sulphonate esters has been pioneered by a study with the azide ion, a very powerful nucleophile. Methyl 2,3-diazido-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside has been prepared by such a replacement on the 3-sulphonate of (II). Azidolysis of a number of other secondary sulphonates have also been investigated. Use of sulphur nucleophiles in these replacements has so far been unsuccessful.

The implications of the work described to the synthesis of α-mercapto-amino sugars is discussed.

## DISCUSSION

A number[1] of chemical compounds possessing a combination of thiol and amino groups have been shown to protect living systems from degradation caused by ionising radiation. One of the most widely studied, and most effective of these compounds is β-mercaptoethylamine.[2] However, the absolute toxicity of this compound restricts the amount which can be used to afford protection. Preparation of a physiologically important compound containing the α-aminomercapto group might give a material with a better protection : toxicity ratio.

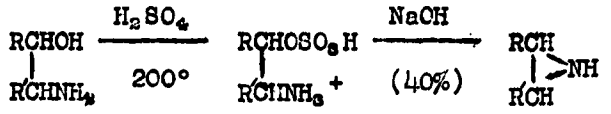
One approach in obtaining non-toxic α-aminomercapto compounds is the placing of this system in ~~which is~~ an otherwise natural molecule, in this case a carbohydrate moiety. Carbohydrates containing this group were hitherto unknown, and it is the aim of this project to explore methods which might be of use in the synthesis of such molecules.

The preparation of potentially useful aminomercapto derivatives of monosaccharides has been undertaken, using two types of nucleophilic reaction, both widely used in carbohydrate chemistry. The reactions which have been studied are: (i) the opening of epoxide rings, and (ii) replacement of sulphonate esters. The reactions are new, however, involving the previously little used $N_3^-$, $SCN^-$, and $PhCH_2S^-$, all powerful nucleophiles. The azide ion has proved to be the most powerful under the conditions used, and has therefore been used to pioneer each new type of reaction.

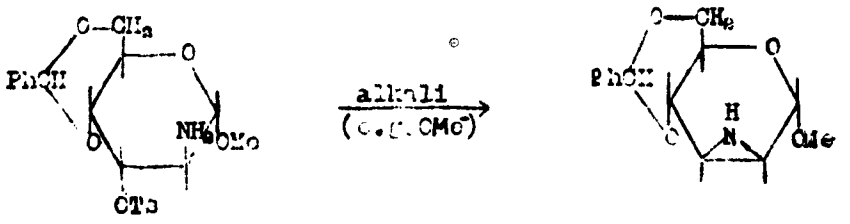
One group of great potential use in the synthesis of α-aminomercapto compounds is the ethylimino system. Such a group would also be useful in the synthesis of diamino sugars.

Christensen and Goodman[3] have described a synthesis of methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-imino-α-D-alloside, via ammonolysis of methyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannoside using a sealed tube. A more convenient synthesis has now been developed.

Ethylenimines have been conveniently prepared[4] from α-amino alcohols via the sulphate. Alkaline hydrolysis of the resulting α-amino sulphate esters gives the imine, with Walden inversion at the carbon atom bearing the oxygen.

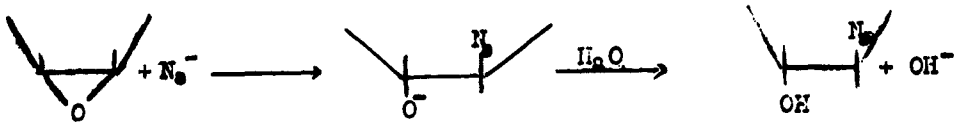


This method should be readily applicable to <u>trans</u> 2-amino or 3-amino sugars, via the toluene-p-sulphonate or the methane-sulphonate ester. Thus for example:



The difficulty of this method is the synthesis of the α-amino, -O-sulphonate ester group. This has been overcome by the introduction of the amino group in the form of an azide derivative, followed by O-sulphonation, and hydrogenation of the azide to $-NH_2$. In this synthesis the azide may be regarded as a conveniently blocked amino group.

Methyl 2,3-anhydro-4,6-O-benzylidene-α-D-alloside,[5] on boiling under reflux for 4 hr. with a four molar excess of sodium azide in 2-methoxyethanol:water (10:1), gave methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altroside (1), (35%), and methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-glucoside (2), (3%). Considerable decomposition was found to occur during the azidolysis; this was presumed to be caused by the alkali liberated in the reaction.



Addition of one mole of ammonium chloride, which should destroy the hydroxyl ions as they are formed, was found to increase the yield

of compound (1) to 75, and (2) to 5% respectively. The ratio of products, obtained by chromatography of the crude syrupy product on alumina was in reasonable agreement with the ratio of di-axial di-equatorial products obtained when the epoxide is opened by other nucleophiles.[6] The two azido sugars were characterised by hydrogenation, in the presence of Adams' catalyst, to the corresponding amino sugars, both of which have been synthesised by other routes.[7,8] The 2-azido altroside (1) was then treated with toluene-p-sulphonyl chloride or methanesulphonyl chloride in pyridine to give the corresponding 3-sulphonates [(3) and (4) respectively]. These were then reduced and subjected to alkaline hydrolysis under a number of conditions in an attempt to obtain methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-imino-α-D-mannoside.

Hydrogenation of the 2-azido-altroside 3-toluene-p-sulphonate (3) in the presence of Adams' catalyst gave the corresponding 2-amino compound. Treatment of this compound with pyridine-acetic anhydride gave the 2-acetamido derivative, which had been synthesised by another route.[9] Treatment of the 2-amino altroside 3-toluene-p-sulphonate with 2.7N methanolic sodium methoxide solution in chloroform at 0°, or with 0.5N methanolic sodium methoxide at reflux gave a crystalline substance (A), which was not the expected imine. Substance (A) had no absorption at 3300 cm.$^{-1}$, due to N-H, as described by Christensen and Goodman[3] for the allo imine, and micro-analysis results indicated that (A) was $C_3H_6$ greater than the expected imine ($C_{14}H_{17}NO_4$).

A similar sequence was carried out aiming at the allo imine described by Christensen and Goodman.[3] Boiling methyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannoside[10] with sodium azide in 2-methoxyethanol-water (10:1) gave only methyl 3-azido-4,6-O-benzylidene-α-D-altroside (5), (68%). No trace of the expected 2-azido-glucoside could be found by chromatography of the crude product. The 3-azido-altroside (5) was readily hydrogenated to the known[11] 3-amino compound in the presence of Adams' catalyst. Treatment of the 3-azido-altroside (5) with toluene-p-sulphonyl chloride in pyridine gave the corresponding 2-toluene-p-sulphonate (6),

hydrogenolysis of which gave a syrup whose infrared spectrum indicated that it contained mainly the 3-amino-altroside 2-toluene p-sulphonate. Treatment of this syrup with boiling 0.5N methanolic sodium methoxide gave a substance (B), analysis of which indicated that it was isomeric with substance (A), and not the expected allo imine of Christensen and Goodman. The analysis and infrared spectra of substances (A) and (B) indicated that they were both methyl 4,6-O-benzylidene-2,3-dideoxy-glycosides, presumably isomers with respect to the orientation of groups about $C_2$ and $C_3$.

Hydrolysis of the 2-amino-altroside 3-sulphonate with 0.5N sodium hydroxide in anhydrous dioxan and in dioxan-water (5:1) gave predominantly substance (A), but a small quantity of syrup was obtained from the mother liquor, which on acetylation using acetic anhydride-pyridine gave a compound whose analysis and infrared spectrum were consistent with its being methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-acetimido-α-D-mannoside (7), i.e. the N-acetate of the required manno imine. Hydrolysis of the 3-amino-altroside 2-toluene-p-sulphonate with 0.5N potassium hydroxide in anhydrous dioxan, followed by treatment of the syrupy product with benzoyl chloride-pyridine, gave methyl 2,3-benzimido-4,6-O-benzylidene-2,3-dideoxy-α-D-alloside (44%) which had been described previously.[3]

Substance (A) was found to be stable to 2N aqueous potassium hydroxide, to sodium azide in boiling DMF-dioxan (5:1), and to hydrogen in the presence of Adams' catalyst. Thus if (A) contains an aziridine ring this must be considerably stabilised. An analysis for =N-Me gave a value of 3.4%, however =N-Me requires 5%.

Treatment of the 2-azido-altroside 3-methanesulphonate (4) with hydrazine hydrate-Raney nickel in boiling methanol gave crystalline methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-imino-α-D-mannoside (8) (81%). The N-acetate (7), identical with that obtained above, and the N-benzoate (9) were readily prepared.

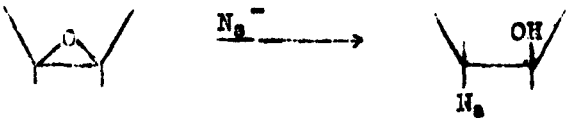
Whereas attempted opening by ammonia of the allo episulphide[12] by Christensen and Goodman gave only a polymeric material, since the intermediate $-S^-$ derivative is a more powerful nucleophile than

ammonia, it is hoped that the imine can be readily opened by such species as $SCN^-$, $RS^-$, and $N_3^-$ to give convenient precursors of 2-amino-3-mercapto, and 2,3-diamino-altrose. Preliminary experiments with $SCN^-$ and $N_3^-$ indicate that this can be achieved.

It is well established[13,14,15] that nucleophilic displacement with Walden inversion occurs during the hydrazinolysis of certain secondary sulphonate esters. This synthetic route has been successfully applied in preparation of 2-amino-2-deoxy-D-ribose,[14] and 2-amino-2-deoxy-L-ribose[15] by Wolfrom and co-workers. The products of such displacements are the comparatively unstable hydrazino derivatives, which must be immediately hydrogenated to give the corresponding amino-sugars Direct ammonolysis[16,17] of sulphonates has been used in similar syntheses, but drastic conditions are necessary.

In view of the unsuitability of anhydrous hydrazine as a nucleophilic reagent attempts have now been made to replace it with the azide ion in these reactions. Azide ion, in a medium of high dielectric constant such as DMF should be a sufficiently powerful nucleophile to displace the sulphonate ion with the production of a stable azide, which can retain its useful properties as a blocked amino group as required.

Methyl 4,6-O-benzylidene-α-D-glucoside 2-toluene-p-sulphonate[10] and sodium azide in boiling 2-methoxyethanol gave methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altroside (5). A similar reaction was observed by Haworth, Hirst and Bodycote,[18] investigating the ammonolysis of the same 2-toluene-p-sulphonate; they obtained the 3-amino-altroside derivative. Peat and Riggins[19] showed that the reaction consisted of two stages, the first being formation of the 2,3-anhydromannoside, which then opened by the reagent.




A similar reaction on the corresponding 2,3-ditoluene-p-sulphonate gave only unchanged starting material. Assuming that in the absence of a neighbouring group effect, the orientation of the sulphonate group has some bearing on the reaction, an axial sulphonate group was next tried. Methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altroside 3-toluene-p-sulphonate (3), and sodium azide in boiling DMF gave only decomposition products. Addition to the system of 20% of dioxan in order to reduce the reaction temperature to 125-135° gave methyl 2,3-diazido-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside (10) in varying yield (1-49%). It was found that a temperature greater than 125° was necessary for the reaction to occur, while above 135° considerable thermal decomposition of the azides occurred. Use of the corresponding methanesulphonate (4) did not substantially alter the yield. The product (10) was assigned the manno configuration since replacement of the sulphonate group on C(3) would be expected[14, 15] to occur with inversion, while the azido group on $C_{(2)}$ should be unaffected. Formation of the diazidomannoside (10) could only be achieved in DMF; this is consistent with the expected $S_N2$ reaction in this type of solvent, in which the organic intermediate is stabilised, but the azide ion concentration is not reduced by solvation.

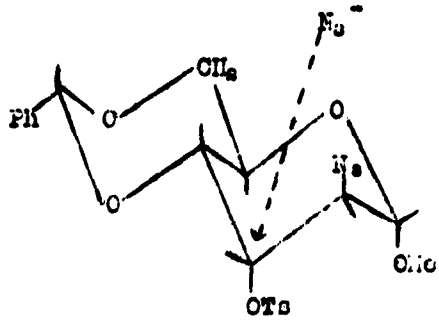
The 2,3-diazido-2,3-dideoxy-mannose derivative (10) was readily hydrogenated in the presence of Adams' catalyst, with formation of a syrupy diamino compound (11), characterised as its crystalline di-acetamido compound. The 2,3-diamino-mannoside (11) consumed 0.96 moles of sodium periodate in 200 minutes, with formation of an insoluble residue which was different from periodate oxidised methyl 4,6-O-benzylidene-α-D-glucoside (12), and from the product obtained by reacting the dialdehyde (12) with ammonia,

During the oxidation, ammonia could be detected by smell; thus it is assumed that the oxidation follows the normal route, as in diols and α-amino alcohols. Ethylenediamine[20] is known to react smoothly with periodate, with formation of formaldehyde.

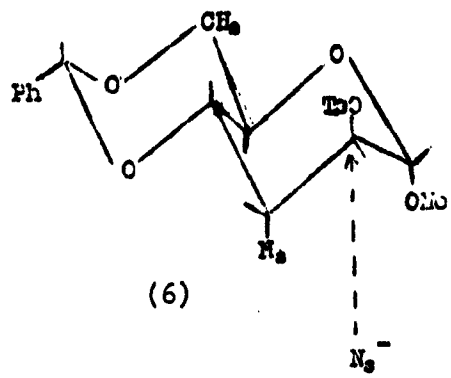
Removal of the benzylidene blocking group from the diazide (10) gave syrupy methyl 2,3-diazido-2,3-dideoxy-α-D-mannoside, which was characterised as its crystalline 4,6-diacetate. It is hoped that removal of the two acetate groups, the glycosidic methyl group, and hyrogenation in the presence of Adams' catalyst will give the free diamino sugar.

Attempts were then made to extend the displacement of sulphonate esters by azide ion to the isomeric 3-azido-altroside 2-toluene-p-sulphonate (6). The reaction with sodium azide in DMF was carried out at a number of temperatures, but only unchanged starting material (89-0°) could be isolated from the mixture. Decomposition increased steadily above 135-140° as in the previous case, and no trace of any diazide could be found.

The failure of this reaction may be due to the stereochemistry of the starting material. Horton, Wolfrom and Thompson[21] studied the hydrazinolysis of methyl 3,5-O-isopropylidene-α-L-xylofuranoside 2-toluene-p-sulphonate and the corresponding methyl β-L-xyloside anomer. Mild hydrazinolysis of the α-L-anomer gave, after reduction and hydrolysis, 2-amino-2-deoxy-α-L-lyxose hydrochloride (33%); the β-L-anomer, on similar treatment gave 2-amino-2-deoxy-β-L-lyxose hydrochloride (2%), and unchanged starting material (82%). This difference was attributed to steric hindrance to the approach of the nucleophile to the back of sulphonate group on $C_{(2)}$. Thus, in the case of the α-anomer the nucleophile must pass between the isopropylidene ring atoms and a hydrogen atom, whereas in the case of the β-anomer the $C_{(1)}$ methoxy group and the isopropylidene ring shield both sides of $C_{(2)}$, greatly hindering the approach of the reagent. The case of the benzylidene locked azido-altroside compounds (3) and (6) is analagous.



(3)



(6)

With the 2-azido-altroside (3) the nucleophile has unhindered access to the back of $C_{(3)}$, passing between the hydrogen atom on $C_{(4)}$, and the axial azide group on $C_{(2)}$. In the 3-azido-altroside (6), the back of $C_{(2)}$ is shielded by the two axial groups on $C_{(1)}$ and $C_{(3)}$. In order to react, azide ion must pass between these two groups, neither of which can effectively move; the difficulty of this readily explains the failure of this attempted displacement.

Other factors must also be considered. The displacement will only occur if the leaving group is <u>axial</u>, due to the unfavourable interactions between axial groups in the pyranose ring. In the 2-azido-altroside 3-toluene-p-sulphonate (3) there is a "1-3 interaction" between the groups on $C_{(1)}$ and $C_{(3)}$, removal of this in a nucleophilic displacement at $C_{(2)}$ will assist the reaction energetically.

Since this work was started Baker[22] and co-workers have reported the displacement of the methanesulphonate group from methyl 2,3,6-tri-O-benzoyl-α-D-galactoside 4-methanesulphonate with sodium azide in boiling DMF to give the corresponding 4-azido-4-deoxy-glucose derivative. Jeanloz[23] has reacted 1,6-anhydro-D-glucose 2,3,4-tritoluene-p-sulphonate with sodium azide in boiling DMF to give the 2,4-diazido-2,4-dideoxy-glucose derivative.

All attempts to secure a nucleophilic displacement in 2-azido-altroside 3-toluene-p-sulphonate (3) using a sulphur containing species have not, so far, been successful. Use of sodium benzyl mercaptide in DMF resulted in immediate decomposition of the azide.

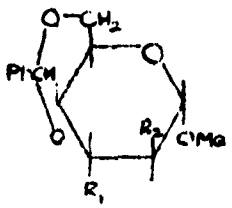
Thiocyanate ion, in boiling 2-methoxyethanol, in boiling DMF and in a eutectic melt of potassium and sodium thiocyanates failed to give any product in which the sulphonate group had been displaced. In each case a varying amount of starting material was isolated, and again thermal decomposition increased sharply above 135-140°.

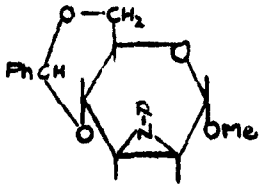
These results are surprising since the accepted order of carbon nucleophilicity places $SCN^- > N_3^-$. However these values are normally determined in systems in which anions are considerably solvated, so that in DMF, which does not solvate any of the anions to an appreciable extent[24] the order may be reversed. In a protic solvent $N_3^-$ is solvated to a greater extent than is $SCN^-$.

Implication of the work described

(i) The use of the azide ion affords an easy method of introducing a blocked amino group into a sugar moeity. This can be done by direct replacement of a sulphonate ester group or by the opening of an epoxide ring.

(ii) The new method of sugar ethylenimine synthesis described makes them readily available. These derivatives are perhaps the best precursors for the synthesis of α-amino-mercapto systems, and it is in this direction particularly that the described studies could be extended.





(1) $R_1$ = OH, $R_2$ = $N_3$.

(3) $R_1$ = OTs, $R_2$ = $N_3$.

(4) $R_1$ = OMs, $R_2$ = $N_3$.

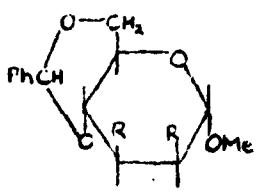
(5) $R_1$ = $N_3$, $R_2$ = OH.

(6) $R_1$ = $N_3$, $R_2$ = OTs.

(7) R = $COCH_3$

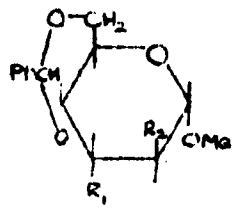
(8) R = H

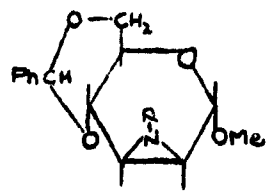
(9) R = $COC_6H_5$



(10) R = $N_3$

(11) R = $NH_2$





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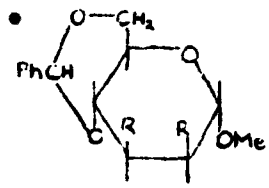

(10) R = $N_3$

(11) R = $NH_2$

## EXPERIMENTAL

All chromatography was on alumina, type 'H' 100-200 mesh, supplied by Peter Spence Ltd.

Rotations were measured in chloroform solution unless otherwise stated.

Where possible compounds were identified by mixed m.p. and by infrared spectroscopy; all new compounds had infrared spectra consistent with the assigned structures.

N,N-dimethylformamide will be referred to as DMF.

All solvents were evaporated using a rotary film evaporator operating at low pressure.

Adams' catalyst was prepared by the method of Adams and Shriner, J. Amer. Chem. Soc., 1923, 45, 2171; Raney nickel by the method of Xorge Alejandro Dominguez, Irma Cavazos Lopez, and Raul France, J. Org. Chem., 1961, 26, 1625.

Azidolyses.

(a) Methyl-2,3-anhydro-4,6-O-benzylidene-α-D-alloside. (i) Sodium azide (3.45 g.) and methyl-2,3-anhydro-4,6-O-benzylidene-α-D-alloside[6] (3.45 g.) in 2-methoxyethanol (40 ml.) and water (5 ml.) were boiled under reflux for 4 hr. The solution was cooled, and poured into ice-water (200 ml.). This mixture was chloroform extracted, the extract dried ($Na_2SO_4$) and evaporated to give a thick syrup, which was chromatographed. Elution was followed polarimetrically; benzene eluted methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altroside (1), (35%), m.p. 79-80°, $[\alpha]_D^{18}$ + 65.0° (C 1.0) (Found: C, 54.8; H, 5.4; N, 13.7. $C_{14}H_{17}N_3O_5$ requires C, 54.7; H, 5.6; N, 13.7%). Elution with benzene-chloroform (1:1) gave methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-glucoside (3%), m.p. 161-162°, $[\alpha]_D^{18}$ + 143° (C 1.0). (Found: C, 54.8; H, 5.5; N, 13.5. $C_{14}H_{17}N_3O_5$ requires C, 54.7; H, 5.6; N, 13.7%).

(ii) Repetition of the above experiment, but with the addition of one molecular proportion of ammonium chloride gave a chloroform extract which was evaporated to give a solid product. Recrystallisation from ethanol gave methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altroside (1) (72%). Chromatography of the mother liquor as above gave more 2-azido altroside (1) (3%), and then methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-glucoside (2), (5%).

The 2-azido-altroside (1) (0.21 g.) in ethanol (50 ml.) was hydrogenated at 1 atm./30° for 20 min. in the presence of Adams' catalyst. There was no net uptake of gas. After removal of the catalyst, the ethanol was evaporated *in vacuo* to give a white solid (0.19 g.), m.p. *ca.* 163°. Recrystallisation from ethanol gave methyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altroside, m.p. 167-168°, $[\alpha]_D^{18}$ + 105° (C 1.0). [lit.[7] m.p. 168°, $[\alpha]_D^{18}$ + 104.7° (C 1.0)].

The 3-azido glucoside (0.2 g.) was hydrogenated as above. The syrupy product was acetylated using pyridine-acetic anhydride to give a white solid (0.13 g.). Recrystallisation from ethanol

gave methyl 3-acetamido-4,6-O-benzylidene-3-deoxy-α-D-glucoside 2-acetate.[8] m.p. 276-278°.

(b) Methyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannoside. The anhydro mannoside[10] (30 g.), sodium azide (30 g.) and ammonium chloride (12 g.) in 2-methoxyethanol (300 ml.) and water (80 ml.) were boiled under reflux for 4 hr. The solution was evaporated and the residue extracted with chloroform. Evaporation gave a semi-crystalline mass, which was extracted with a little boiling ethanol. Cooling gave large transparent cubes (13.9 g.), m.p. 135-136°. The mother liquor was evaporated to give a brown syrup which was chromatographed. Elution with chloroform gave a further crop (9.9 g.), m.p. 135-136°. The combined crops were recrystallised from ethanol to give methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altroside (5) (23.8 g., 69%), $[\alpha]_D^{18}$ + 39.9° (c 1.00) (Found: C, 54.4; H, 5.5. $C_{14}H_{17}N_3O_5$ requires C, 54.7; H, 5.6%). Elution with ethanol gave methyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-altroside (1%) m.p. 185-186°.

The 3-azido altroside (5), (0.5 g.) in methanol (50 ml.) was hydrogenated at 1 atm. and at room temperature using Adams' catalyst (0.1 g.), for 15 hr. The catalyst was removed and the solvent evaporated to give a white crystalline solid (0.52 g.). Two recrystallisations from ethanol gave methyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-altroside, (0.36 g.) m.p. 186-187° (lit.[11]: m.p. 184-185°).

(c) 1,2-O-Isopropylidene-D-xylose 5-toluene-p-sulphonate. The xylose 5-toluene-p-sulphonate (1.72 g.) in 2-methoxyethanol (25 ml.) was added to sodium azide (1.3 g.) in water (10 ml.) and the solution boiled under reflux for 24 hr. Evaporation in vacuo gave a semi-crystalline mass which was extracted with dry acetone, leaving inorganic residues (1.38 g.). Evaporation of the acetone yielded a brown syrup (0.87 g.) which crystallised to give straw coloured needles on leaving overnight in a desiccator. This solid could not be recrystallised from any solvent. After 10 days in the vacuum desiccator the needles ($\nu N_3$ 2090 cm.$^{-1}$) had m.p. ca. 55°.

(d) Methyl 4,6-O-benzylidene-α-D-glucoside 2-toluene-p-sulphonate. The glucoside-2-toluene-p-sulphonate[10] (10 g.) in 2-methoxyethanol (100 ml.) was added to sodium azide (10 g.) in water (20 ml.), and the solution boiled under reflux for 24 hr. Hot water was added to turbidity, followed by cooling to give unchanged starting material (36%), m.p. 148-149°. The mother liquor was diluted with cold water (200 ml.) and then chloroform extracted. The extract was well washed with water, and dried ($Na_2SO_4$). Evaporation gave a syrup which on crystallisation from ethanol gave methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altroside (5), (17%), m.p. 135-136°. Nothing further could be isolated from the mother liquors.

(e) 1,2:5,6-Di-O-isopropylidene-D-glucose 3-toluene-p-sulphonate. Diacetone glucose 3-toluene-p-sulphonate[16] (6 g.) and sodium azide (6 g.) in 2-methoxyethanol (100 ml.) and water (20 ml.) were boiled under reflux for 48 hr. The solution was then saturated by addition of hot water. Cooling gave unchanged starting material (97%), m.p. 119-120°.

(f) Methyl 4,6-O-benzylidene-α-D-glucoside 2,3-ditoluene-p-sulphonate. The glucoside ditoluene-p-sulphonate,[26] (10 g.) in 2-methoxyethanol (100 ml.), and sodium azide (10 g.) in water (5 ml.) were mixed with DMF (20 ml.) and the whole boiled under reflux for 60 hr. The mixture was poured into cold water. The white syrupy mass, which was precipitated, was filtered off and washed with water. Crystallisation from ethanol-acetone (1:1) gave unchanged starting material (87%), m.p. 153-154°. No other substance could be isolated from the mother liquors.

Methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altroside 3-toluene-p-sulphonate. The 2-azido altroside (1), (10 g.) in pyridine (50 ml.) containing toluene-p-sulponyl chloride (20 g.) was left at room temperature for 4 days. After hydrolysis of excess toluene-p-sulphonyl chloride with a little water the solution was poured into ice-water (200 ml.); the solid product was well washed with water, and dried at 65°. Recrystallisation from ethanol-acetone

(1:1) gave methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altroside 3-toluene-p-sulphonate, (3) (15.1 g.; 99%), m.p. 189-190° (decomp.), $[\alpha]_D^{20}$ + 56.2°, (C 1.0) (Found: C, 55.0; H, 4.9. $C_{21}H_{23}N_3O_7S$ requires C, 54.7; H, 5.0%).

Methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altroside 3-methanesulphonate. The 2-azido altroside (1), (2.5 g.) and methane sulphonyl chloride (1.2 ml.) in pyridine (8 ml.) were mixed at 0°, and then kept overnight at room temperature. After hydrolysis of the excess methane sulphonyl chloride, the solution was poured into ice-water. This mixture was chloroform extracted, and the extract washed with cold dilute hydrochloric acid, cold water, and then dried ($Na_2SO_4$). Evaporation in vacuo gave a yellow glass (3.2 g.). Crystallisation and then recrystallisation from ethanol gave white plates of methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altroside 3-methanesulphonate, (1.8 g., 54%), m.p. 152-153°, $[\alpha]_D^{20}$ + 39.0° (C 1.0). (Found: C, 47.2; H, 5.0. $C_{15}H_{19}N_3O_8S$ requires C, 46.8; H, 5.0%).

Methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altroside 2-toluene-p-sulphonate. The 3-azido-altroside (5) was treated with toluene-p-sulphonyl chloride as described for the 2-azido derivative. The crude product, on recrystallisation gave methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altroside 2-toluene-p-sulphonate, (8.6 g., 96%), m.p. 117-118°, $[\alpha]_D^{18}$ + 26.2° (C 1.03), (Found: C, 55.1; H, 4.9. $C_{21}H_{23}N_3O_7S$ requires C, 54.7; H, 5.0%).

Methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altroside 2-methanesulphonate. This was prepared as described above for the corresponding 2-azido-3-methanesulphonate derivative. The white solid, on recrystallisation from methanol, gave methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altroside 2-methanesulphonate, (79%) m.p. 130-131°, $[\alpha]_D^{20}$ + 9.3° (C 1.12), (Found: C, 46.5; H, 4.7. $C_{15}H_{19}N_3O_7S$ requires C, 46.8; H, 4.5%).

Methyl 2-azido-2-deoxy-α-D-altroside. The 2-azido altroside (1) (4 g.) in 60% acetic acid (50 ml.) was refluxed until all of the solid had dissolved (2 hr.). The acetic acid and benzaldehyde were removed by co-distillation with water in vacuo, and the

solution was then evaporated to give a white solid (3.42 g.). Two recrystallisations from ethanol gave methyl 2-azido-2-deoxy-α-D-altroside, m.p. 140-141°, $[\alpha]_D^{20}$ + 63.8°. (C 1.05 in methanol). (Found: C, 38.5; H, 6.2. $C_7H_{13}N_3O_6$ requires C, 38.4; H, 6.0%).

Methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altroside 2-acetate. The 3-azido-altroside (5), (5 g.) in pyridine (50 ml.) was treated with acetic anhydride (3 g.) and the mixture kept at room temperature for 30 hr. The solution was poured into ice-water (500 ml.) and the precipitate twice recrystallised from ethanol to give methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altroside 2-acetate, (4.6 g.), m.p. 122-123°, $[\alpha]_D^{20}$ + 20.8° (C 1.2).

Methyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altroside 3-toluene-p-sulphonate.

(a) The 2-azido altroside-3-toluene-p-sulphonate (1.7 g.) in methanol (60 ml.) and chloroform (30 ml.) was hydrogenated at 1 atm. and at room temperature in the presence of Adams' catalyst. Gas was slowly taken up over 1 hr. The catalyst was filtered off, and the solvent removed *in vacuo* to give a white solid which was recrystallised from methanol, yielding unchanged starting material (98%), m.p. 199-200° (decomp.).

(b) The 2-azido-altroside-3-toluene-p-sulphonate (1.5 g.) in methanol (50 ml.) was hydrogenated at 1 atm. and at room temperature using Adams' catalyst. After 50 hr. the catalyst was removed and a white foaming syrup (1.6 g.) was obtained by evaporation *in vacuo*. The solvent could not be completely removed by evaporation, hence weight of syrup always exceeded the theoretical amount. Bands at 3400 and 1647 $cm.^{-1}$ in the infrared suggested that the syrup consisted mainly of methyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altroside 3-toluene-p-sulphonate (3). This was confirmed by acetylation (pyridine-acetic anhydride). The white product was twice recrystallised from ethanol to give methyl 2-acetamido 4,6-O-benzylidene-2-deoxy-α-D-altroside 3-toluene-p-sulphonate, (41% based on the azide) m.p. 174-175°, $[\alpha]_D^{18}$ + 61° (C 1.0, acetone). [lit.[9]: m.p. 174°, $[\alpha]_D^{18}$ + 62°, (C 1.0, acetone)]

Attempted preparation of methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-imino-α-D-mannoside.

(a) A solution of the syrupy 2-amino-altroside [from 5.5 g. (3),] in chloroform (100 ml.) was cooled to 0° and treated with 2.7N. methanolic sodium methoxide (25 ml.). The solution was kept 3 days at 0°, 1 day at room temperature, and then washed with water until neutral, dried ($Na_2SO_4$), and evaporated to give a white solid. Two recrystallisations from ethanol gave white needles of substance (A), (2.05 g.), m.p. 106-107°, $[\alpha]_D$ + 59.9° (C 0.84). (Found: C, 66.6; H, 7.8; N, 4.6%). The above hydrolysis could not be repeated.

(b) A solution of the syrupy 2-amino-altroside (from 10 g. (3), in 0.5N. methanolic sodium methoxide (200 ml.) was boiled under reflux for 2 hr. The solution was then poured into ice-water (600 ml.). The white crystalline precipitate was collected and washed with water (500 ml.). Recrystallisation from aqueous methanol gave substance (A), (5.02 g.) as fine white needles, m.p. 106-107°, $[\alpha]_D$ + 59.9° (C 0.84). (Found: C, 67.3; H, 7.4%).

(c) Repetition of (b), but with chloroform extraction of the ice-water: product mixture gave, after washing, drying ($Na_2SO_4$), and evaporating the extract, a yellow syrup which could not be crystallised. Acetylation with pyridine-acetic anhydride gave a white solid, which on recrystallisation from ethanol, gave white crystals (1.4 g.), m.p. 205-206° (decomp.), $[\alpha]_D^{18}$ + 49.3° (C 0.69), believed to be methyl 2,3-acetimido-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside (7). The product had no N-H or O-H absorbtion, and only one strong band at 1710 cm.$^{-1}$ attributed to $N\text{-}COCH_3$ in the infrared spectrum. The above could not be repeated.

(d) The syrupy 2-amino-altroside (from 10 g. of the azide (3), in dioxan:water (5:1), (250 ml.), was treated with potassium hydroxide (7 g.), and the solution boiled under reflux for 2 hr. Pouring into ice-water (1 l.) gave a white precipitate, which on two recrystallisations from aqueous ethanol gave substance (A), (4.5 g.)

m.p. 105-107°. Chloroform extraction of the mother liquor gave a small amount of a syrup, which on treatment with acetic anhydride-pyridine gave methyl 2,3-acetimido-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside (7) (0.21 g.), m.p. 205-206° (decomp.).

(e) The syrupy 2-amino-altrodide (from 4.5 g. of the azide (3), in 2-methoxyethanol (50 ml.) was treated with hydrated sodium acetate (4 g.), and the solution boiled under reflux for 24 hr. The solution was poured into water (500 ml.) and the precipitate twice recrystallised from aqueous ethanol to give substance (A) (1.19 g.) m.p. 106-107°. No other product could be isolated.

(f) The 2-azide-altroside 3-methane sulphonate (4), (15 g.) in methanol (200 ml.) was treated with hydrazine hydrate (15 g.). Raney Ni was added, and the mixture boiled under reflux until decomposition of the hydrazine was complete (2.5 hr.). Filtration and evaporation gave a white solid, m.p. 110-116°. Recrystalliaation from aqueous methanol gave methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-imino-α-D-mannoside (3.47 g.) m.p. 145-146°, $[\alpha]_D^{20}$ + 22.8°, (C 1.14), (Found: C, 63.8; H, 6.6; N, 5.4. $C_{14}H_{17}NO_4$ requires C, 63.9; H, 6.5; N, 5.3%).

Treatment of the imino-mannoside with acetic anhydride-pyridine gave methyl 2,3-acetimido-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside, m.p. 205-206° (decomp.), $[\alpha]_D^{18}$ + 49.3°, (C 0.69). (Found: C, 62.8; H, 6.3; N, 4.6. $C_{16}H_{19}NO_5$ requires C, 62.9; H, 6.3; N, 4.6%).

Reaction of the imino with benzoyl chloride-pyridine gave methyl 2,3-benzimido-4,6-O-benzylidene 2,3-dideoxy-α-D-mannoside, (70%), m.p. 165-166°, $[\alpha]_D^{20}$ + 5.8° (C 0.87). (Found: C, 68.8; H, 5.8. $C_{21}H_{21}NO_5$ requires C, 68.7; H, 5.8%).

Reactions of Substance (A). A suspension of substance (A), (1.0 g.) in 1.2N aqueous potassium hydroxide (40 ml.) was boiled under reflux for 24 hr. The reaction mixture was cooled to 0° and filtered. The white solid was recrystallised from aqueous methanol to give substance (A), (0.99 g.), m.p. 106-107°.

Substance (A) (0.5 g.) in methanol (100 ml.) was hydrogenated using Adams' catalyst at 1 atm. and at room temperature for 3 hr.

There was only a negligable uptake of hydrogen. Removal of the catalyst and evaporation gave unchanged (A), (0.35 g.), m.p. 107-108°.

Substance (A) (0.9 g.) and sodium azide (1.0 g.) in DMF (20 ml.) and dioxan (5 ml.) were boiled under reflux for 8 hr. (130°). The solution was poured into ice-water (300 ml.), and the precipitate recrystallised from aqueous ethanol to give unchanged (A), (0.6 g.), m.p. 107-108°.

Methyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-altroside 2-toluene-p-sulphonate. The 3-azido altroside 2-toluene-p-sulphonate (6), (10 g.) in methanol (200 ml.) was hydrogenated at 1 atm. and at room temperature using Adams' catalyst. The catalyst was removed and the solution evaporated *in vacuo* to give a white foam (11 g.), whose infrared spectrum was that expected for methyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-altroside 2-toluene-p-sulphonate.

Hydrolysis of the 3-amino-altroside-2-toluene-p-sulphonate. The solution of the syrupy 3-amino altroside [from 10 g. (6)] in 0.5N methanolic sodium methoxide (200 ml.) was boiled under reflux for 2 hr., and then the solution was poured into cold water (200 ml.). The white precipitate was collected and recrystallised from aqueous methanol to give substance (B) (4.75 g.), m.p. 132-133°, $[\alpha]_D^{20}$ + 140° (C 1.18). (Found: C, 66.8; H, 7.2%).

Analysis and infrared spectra indicated that (B) was isomeric with (A), presumably with respect to orientation of groups about $C_{(2)}$ and $C_{(3)}$.

Methyl 2,3-diazido-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside.

The 2-azido altroside 3-toluene-p-sulphonate (3 g.) and sodium azide (3 g.) in 2-methoxyethanol (20 ml.) and water (4 ml.) were boiled under reflux for 24 hr. The solution was then poured into water (200 ml.). The grey precipitate was recrystallised from ethanol-acetone (1:1) to give unchanged starting material (88%), m.p. 189-190° (decomp.).

The 2-azido-altroside-3-toluene-p-sulphonate (2.22 g.) and

sodium azide (3 g.) in DMF (50 ml.) were boiled under reflux for 48 hr. The resulting black solution was poured into cold water (300 ml.). The black semi-crystalline mass which separated was filtered off, taken up in acetone (100 ml.) and boiled under reflux with active charcoal for 1 hr. Filtration gave a clear solution, which on evaporation gave a white solid. Washing of the charcoal with boiling acetone gave no more product. Two recrystallisations from ethanol gave white needles of methyl 2,3-diazido-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside (0.03 g.) (1%), m.p. 151-152° (decomp.), $[\alpha]_D^{18}$ + 107° (c 0.67) (Found: C, 50.6; H, 6.6; N, 25.2. $C_{14}H_{16}N_6O_4$ requires C, 50.6; H, 6.5; N, 25.3%).

The 2-azido-altroside 3-toluene-p-sulphonate (3), (1.5 g.) and sodium azide (1.5 g.) in DMF (20 ml.) and dioxan (5 ml.) were boiled under reflux for 48 hr. (125-130°). The black solution was poured into ice-water (400 ml.), and the precipitate filtered off, dissolved in dry acetone (50 ml.) and boiled for 10 min. with active charcoal. Filtration, evaporation and recrystallisation of the residue from ethanol gave the 2,3-diazido-mannoside (0.51 g., 49%), m.p. 151-152°.

A large scale preparation (80 g. of starting material) gave a yield of 22%.

Repetition of the above, but using the 2-azido-altroside 3-methanesulphonate (5 g.) gave the diazido mannoside (43%).

The 2,3-diazido-mannoside (2 g.) in methanol (100 ml.) was hydrogenated for 1 hr. at 1 atm. and at room temperature in the presence of Adams' catalyst. Removal of the catalyst and evaporation gave a thick syrup, (1.95 g.), $[\alpha]_D^{19}$ + 29.1°, (c 0.89), whose infrared spectrum suggested that it consisted mainly of methyl 2,3-diamino-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside.

The syrupy diamino-mannoside (1.49 g.) was treated with acetic anhydride in pyridine. After 30 min. the solution was poured into water, and the mixture chloroform extracted. The chloroform extract was washed with cold 2N hydrochloric acid and with sodium bicarbonate solution, dried ($Na_2SO_4$), and evaporated to give a white

solid. Two recrystallisations from ethanol gave methyl 2,3-diacetamido-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside, (1.13 g.) m.p. ca 303° (decomp.), 310-311° (preheated to 300°), $[\alpha]_D^{20}$ - 36.2° (C 0.99 in DMF). (Found: C, 59.5; H, 6.7. $C_{17}H_{24}N_2O_6$ requires C, 59.3; H, 6.6%).

Periodate oxidation of methyl 2,3-diamino-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside.

(a) The syrupy diamino-mannoside (0.0284 g.) in methanol (5 ml.) was treated with a solution of sodium metaperiodate (5 ml., 30.29 x $10^{-3}$M.). The rotation of the mixture fell to a constant value after 130 min.

(b) The syrupy diamino-mannoside (0.0811 g.) in water (50 ml.) was treated with a solution of sodium metaperiodate (50 ml., 30.29 x $10^{-3}$M.) Analysis of the reaction by the method of Muller and Friedburger showed that the diamino compound had taken up 0.96 moles of periodate in 200 min.

| Time (min.) | 0 | 15 | 30 | 60 | 200 |
|---|---|---|---|---|---|
| Periodate uptake (moles) | 0 | 0.69 | 0.78 | 0.90 | 0.96 |

(c) The syrupy diamino compound (0.5 g.) in water (200 ml.) was treated with sodium metaperiodate (0.8 g.). The mixture was left in the dark overnight, and the white precipitate (0.15 g.) removed by filtration. This material was found to be different from periodate-oxidised methyl 4,6-O-benzylidene-α-D-glucoside, and the product obtained by treating this dialdehyde with ammonia.

Methyl 2,3-diazido-2,3-dideoxy-α-D-mannoside. The diazido-benzylidene-mannoside (2.5 g.) in 60% aqueous acetic acid (50 ml.) was boiled under reflux for 30 min. Acetic acid and benzaldehyde were removed by distillation, and the solution evaporated to give a thick syrup (2.41 g.), $[\alpha]_D^{20}$ + 108°, (C 1.03 in MeOH). The infrared spectrum was consistant with that expected for methyl 2,3-diazido-2,3-dideoxy-α-D-mannoside. Attempts to crystallise the syrup from the common solvents were all unsuccessful.

The syrupy methyl 2,3-diazido-2,3-dideoxy-α-D-mannoside (0.5 g.) was treated with pyridine-acetic acid, and the solution left at room temperature overnight. Working up in the usual way gave a

white solid, (0.38 g.). Two recrystallisations from aqueous ethanol gave methyl 2,3-diazido-2,3-dideoxy-α-D-mannoside 4,6-diacetate, m.p. 82-83°, $[\alpha]_D^{20}$ + 114°, (C 0.93). (Found: C, 40.2; H, 4.9. $C_{11}H_{16}N_6O_6$ requires C, 40.3; H, 5.0%).

Azidolysis of methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altroside 2-toluene-p-sulphonate.

(i) The 3-azido-2-toluene-p-sulphonate (6), (1.5 g.) and sodium azide (1.5 g.) in DMF (50 ml.), and dioxan (20 ml.) were boiled under reflux at 125° for 48 hr. Pouring into water gave a white semi-crystalline mass which on crystallisation from methanol gave unchanged starting material (89%), m.p. 116-117°.

(ii) The above was repeated using DMF-dioxan in different ratios, all gave unchanged starting material m.p. 116-117°.

| DMF-dioxan | 5:1 | 10:1 | 100:1 |
|---|---|---|---|
| Unchanged starting material (%) | 62 | 21 | 1 |

(iii) Repetition of the above, but using dimethyl sulphoxide as the solvent gave a black solution from which no product could be isolated.

(iv) Repetition of experiment (i) above, using the 3-azido-2-methanesulphonate derivative gave only unchanged starting material (30%), m.p. 130-131°.

Attempted synthesis of sulphur derivatives.

(i) Benzthiolation of methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altroside 3-toluene-p-sulphonate. The 2-azido-3-toluene-p-sulphonate derivative (2.3 g.) in DMF (100 ml.) was treated with a solution of sodium (1.35 g.) in methanol (10 ml.) and benzyl mercaptan (3.2 g.). The mixture was boiled under reflux for 18 hr. in an atmosphere of nitrogen. The solution was poured into ice-water (800 ml.) and the mixture chloroform extracted. Working up the chloroform in the usual way gave no product which could be characterised.

(ii) Action of the thiocyanate ion on methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altroside 3-toluene-p-sulphonate. The 2-azido-3-toluene-p-sulphonate (1 g.) and ammonium thiocyanate (2 g.) in DMF (20 ml.) were boiled under reflux for 48 hr. Working up as

above gave no product which could be characterised.

(iii) The 2-azido-altroside-3-toluene-p-sulphonate (1.5 g.) was added to a molten mixture of sodium thiocyanate (10 g.) and potassium thiocyanate (40 g.) at 150°. An immediate evolution of gas indicated that thermal decomposition of the azide had occurred. After 6 hr. the mixture was extracted with water; no insoluble residue remained.

(iv) Repetition of (ii) above, but using the 2-azido-3-methane sulphonate derivative in boiling DMF-dioxan (5:1) gave, on working up, only unchanged starting material (52%), m.p. 130-131°.

## ADMINISTRATION

Mr. D. Murphy has been employed full-time on this project, under the direction of Dr. R. D. Guthrie. The following is an approximation of the man-hours, expended on the project:

Dr. R. D. Guthrie .. .. approximately 300 man. hours

Mr. D. Murphy .. .. .. approximately 2000 man hours

The only materials expended on the contract have been chemicals, general laboratory apparatus, and microanalyses. Where necessary some of the University's equipment was used for the contract, but no charge has been made.

## PUBLICATION

" Synthesis of 2,3-Diamino-2,3-dideoxy-D-mannose Derivatives via Azido-sugar Intermediates" , Chemistry and Industry, 1962, 1473.

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